Low temperature wheat germplasm and its leaf photosynthetic traits and structure characteristics*

ZHANG Songwu**, MIAO Fang and WANG Changfa

(Northwest Sci-Tech University of Agriculture and Forestry, Yangling 712100, China)

Received September 17, 2003; revised November 10, 2003

Abstract Low temperature germplasm with constant low plant temperature was found in the nature through a long-time observation on wheat canopy temperature and traits; correspondingly, high temperature germplasm with constant high plant temperature also exists. Compared with the high temperature germplasm, the chlorophyll content and the net photosynthetic rate of the three functional leaves on the top of the low temperature wheat germplasm are higher and the structure tends to be more complicated, which is characterized by smaller mesophyll cells and more closely arranged cell layers, more and denser chloroplasts with thick stroma, more granas and well developed grana lamellae, a larger vascular bundle area with smaller interspace. All these characteristics embody the consistency of structure and function and provide the theoretical bases for looking for and cultivating the new low temperature materials in agricultural practice.

Keywords: **low temperature wheat germplasm, leaf, photosynthetic trait, structure.**

In the past twenty years, low temperature germplasm materials have been found in Gramineae, Leguminosae and Solanaceae due to the application of advanced infrared temperature measurement techniques[1~13]. These materials are characterized by the constant low temperature and some favorable functional and structural traits, which attracted great attention from researchers. Since 1984, we have studied low temperature materials, mainly focused on wheat. During the research, we met with a problem concerning the definition of threshold temperature materials, which still has no universal definition. Therefore a definition of low temperature wheat germplasm was proposed: it refers to the wheat germplasm with similar or constant lower canopy temperature (plant temperature) during the grain filling and maturation stage (flowering-maturation) when compared with long-cultivated local wheat varieties in a small area (such as an experimental field) with the same climate and soil conditions and cultivation measures in an agricultural ecological region. Contrarily, the high temperature germplasm is referred to as those materials with constant higher canopy temperature. In fact, the temperature difference between different genotypes appears before flowering, but less obvious than that after the blooming period. This paper will mainly address the leaf photosynthesis traits and the related micro- and ultrastructure of low temperature wheat germplasm; and clarify the characteristics of low temperature wheat germplasm in terms of the wheat growth and development.

1 Materials and methods

1.1 Experimental design

The experimental plot lies in the experimental station of the Northwest Sci-Tech University of Agriculture and Forestry in the uppermost terrace area of Weihe River valley in Shaanxi Province, which is a part of the most important wheat production area—winter wheat production area of Huanghuai Plain in China. It is the warm temperate semihumid climate.

Hundreds of materials of wheat have been used in the experiment and chosen from time to time. Among them the representative materials (about 15) have been systemically observed for years. Xiaoyan 6, the control plant in this research, was derived from the interspecific hybridization and has been grown more than twenty years in local practice. It is characterized by a high and steady yield and good quality. The planting specifications in the plot are as follows: the plot is free of stub before planting; the seeds are randomly disposed in blocks with 3 repeats in the first ten days of October every year (the best time for sowing); and there are seven rows (2.67 m long) per block with row spacing of 0.25 m and plant spacing of

* Supported by the National Natural Science Foundation of China (Grant Nos. 30070150 and 30270766)
** To whom correspondence should be addressed. E-mail: coldwheat@hotmail.com

0.03 m; the field has been managed to meet the requirements of comparative experiment on varieties in Huanghuai Plain winter wheat zone.

1.2 Observations

The canopy temperature of experimental materials has been observed and recorded for more than a decade in the symmetrical way used in farmland microclimate measurement. The observation was made in sunny afternoon (13 pm ~ 15 pm) when the canopy temperature of experimental materials differs greatly. Great care should be taken in choosing the most representative points, avoiding the influence of bare ground. The infrared thermometer BAU-I with the resolution of 0.1 ℃ and the accuracy of ±0.2 ℃ was used for measurement of the canopy temperature; its response time is 2 ~ 3 s, and the visual field angle is 5°.

The chlorophyll content was measured with the spectrophotometer UVIKON810; and the net photosynthesis rate was measured with the portable photosynthesis measurement system LI-6400 (LI-COR, USA).

The leaf structure was observed at different stages of the growth process, which include flowering stage, grain formed stage, early milky stage, middle milky stage, and late milky stage. At every stage, one plant was selected from each block (three for each material), and the three leaves on the top of the main stem were taken. A 1 mm^2 area on the leaves was chipped off, and fixed by glutaradehyde and osmic acid, and then embedded in Epon812 mixed embedding medium. The embedded samples were sliced into sections of 1 ~2 μm with an ultramicrotome and dyed with the totuidine blue for the microstructure observation under the optical microscope. Meanwhile, ultrathin sections of 60 nm were made with routine methods and the ultrastructure of the leaf samples was observed under the 100CX-II transmission electron microscope. Microstructure observation of 30 repeats was required for each structure index of each leaf, and statistical analysis was made. Ultrastructure observation was required to count grana stacks and lamellae of 100 granas for each leaf.

All observations and measurements were focused on the grain filling and maturation stage that is crucial to the final yield. Three low and 3 high temperature germplasm materials were involved in the experiment. Similar traits were observed in the same temperature germplasm materials while there existed obvious difference between the low and high temperature germplasm materials. In order to clarify it, the typical low temperature germplasm Shaan229 and the typical high temperature germplasm NR9405 were selected and compared, both of which have a similar average height of about 78 cm, the same loose plant form, moderate stooling and only a 1-day difference in their maturation periods. Some projects have included the results from low temperature germplasm 901 and Xiaoyan 6 and high temperature germplasm 9430 and Yanshi9. These materials fall into the local middle maturation type and exhibit only a 1- to 3-day difference in their maturation periods.

2 Results and analysis

2.1 The low temperature phenomenon of wheat

The canopy temperature data of the low temperature germplasm Shaan229, the high temperature germplasm NR9405 and the control material Xiaoyan6 during the grain filling and maturation stage from 1995 to 1999 are plotted (in Fig. 1). The x-axis represents day series (15 observation days), and the y-axis represents temperature difference, namely the temperature differences among the low and high temperature germplasm and the control.

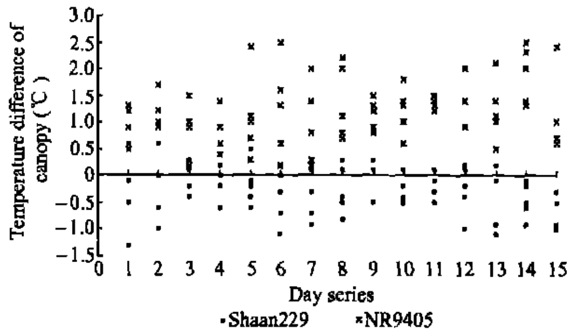

Fig. 1. Canopy temperature of the low and high temperature germplasms in grain filling and maturation stage.

Fig. 1 shows that most canopy temperature difference values of Shaan229 are near or below the base line (the temperature difference line of 0.0 ℃) while those of NR9405 are far above it. The difference is quite obvious. Statistical analysis indicates that the temperature difference between the low and high temperature germplasms reaches a significant level ($t > t_{0.05}$) in the first 4 observation days (possibly influenced by the absence of sample data from 1999), and a very significant level ($t > t_{0.01}$) on other observation days. The temperature of the low temperature germplasm is low not only in normal years but also in drought and waterlogged years. This is the low temperature phenomenon of wheat that attracted great attention. The following analysis of leaf photosynthetic function and structure provides great insight into this low temperature phenomenon.

2.2 Photosynthetic traits and structure of the leaf

2.2.1 Chlorophyll content

Table 1 shows the changes in chlorophyll content in the three top leaves of the low and high temperature germplasms in the grain filling and maturation stage. The contribution to grain influx of the leaves in their different locations has been included in the weighted average.

Table 1. Chlorophyll content of low and high temperature germplasm leaves during grain filling and maturation stage ($mg \cdot g^{-1}$ FW)

Materials	Leaf location	Date									
		05-03	05-06	05-09	05-12	05-15	05-18	05-21	05-24	05-27	05-30
Shaan229	Flag leaf	3.883	4.130	4.377	4.477	4.576	4.226	4.253	4.279	2.765	2.469
	2nd top leaf	2.913	3.209	3.280	3.402	3.366	3.329	3.255	3.181	2.741	2.301
	3rd top leaf	2.285	2.313	2.340	2.532	2.447	2.354	2.260	1.687	1.490	1.293
	Weighted average	3.344	3.566	3.727	3.849	3.880	3.659	3.638	3.533	2.538	2.220
NR9405	Flag leaf	3.502	4.054	3.036	2.753	2.664	2.487	2.341	0.797	0.522	0.059
	2nd top leaf	2.053	3.007	2.559	2.001	1.871	1.448	0.982	0.437	0.000	0.000
	3rd top leaf	1.497	2.168	1.166	1.321	1.475	1.424	0.496	0.242	0.000	0.000
	Weighted average	2.762	3.444	2.584	2.302	2.243	2.021	1.653	0.603	0.290	0.033

Table 1 indicates that at the same leaf location on the same day, leaf chlorophyll content of Shaan229 is higher than that of NR9405. Moreover, peak leaf chlorophyll content of Shaan229 occurs near the middle milky stage, which is later than that of NR9405. As grain approaches maturity, chlorophyll of the 2nd and 3rd top leaves of high temperature germplasm decomposes completely, while some chlorophyll still remains in leaves of different leaf locations of low temperature germplasm. Table 1 also indicates a similarity between Shaan229 and NR9405: the chlorophyll content decreases with descending of leaf location through each growth stage. Statistical analysis of the leaf chlorophyll content at the same leaf location and the weighted average of leaf chlorophyll content of the top three leaves of Shaan229 and NR9405 on the same day shows that $S = \min(n+, n-) = 0$, while $S_{10, 0.01} = 0$. That is, $S \leqslant S_{10, 0.01}$, indicating a considerable difference between chlorophyll contents in the top three leaves of the two germplasms.

2.2.2 Net photosynthetic rate

Fig. 2 shows the change in net photosynthetic rate of the three top leaves of low and high temperature germplasms during the grain filling and maturation stage.

It indicates that the net photosynthetic rates of the low and high temperature germplasm leaves tend to decrease with time during the gain filling and maturation stage. On every observation day in both germplasms, relative net photosynthetic rate was observed as: flag leaf > 2nd top leaf > 3rd top leaf. Comparison of the two germplasms shows that, at the same leaf location, the net photosynthetic rate of the low temperature germplasm is always higher than that of the high temperature germplasm. Statistical analysis indicates that $S = \min(n+, n-) = 0$ and $S_{6, 0.05} = 0$, such that $S \leqslant S_{6, 0.05}$. This indicates that the difference in net photosynthetic rate between leaves at each leaf location on the low and high temperature germplasms is considerable. Since the net photosynthetic rate reflects the potential ability of the leaf to produce and transport photosynthetic sub-

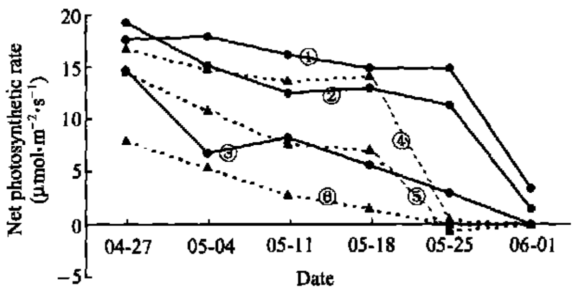

Fig. 2. Net photosynthetic rate of low and high temperature germplasm leaves during the grain filling and maturation stage. ① Flag leaf of Shaan229, ② 2nd top leaf of Shaan229, ③ 3rd top leaf of Shaan229, ④ flag leaf of NR9405, ⑤ 2nd top leaf of NR9405, ⑥ 3rd top leaf of NR9405.

stances, the higher rate of net photosynthesis in the low temperature germplasm has important implications for wheat cultivation.

2.2.3 Mesophyll cell, chloroplast and chloroplast grana lamellae

Leaf cross-sections (Fig. 3 (a) and (b)) show that, in comparison with the high temperature germplasm, the mesophyll cells of the low temperature germplasm are smaller and more closely arranged, and have less intercellular space and more cell layers. Also, the volume of the chloroplast in mesophyll cells in the low temperature germplasm is comparatively large, and its short diameter is obviously long. The chloroplasts are arranged more closely along cell walls, in double or multiple layers (Fig. 3 (a) and (c)). However, the chloroplasts in mesophyll cells of the high temperature germplasm have a small volume and are less closely arranged in a single layer (Fig. 3 (b) and (d)). In the top three leaves, the mesophyll cells in unit leaf area increase with ascending of leaf location. The average number of the chloroplasts per mesophyll cell in the flag leaf, the 2nd and 3rd top leaves is 23.0, 20.7, 17.2 respectively in Shaan229 and 16.4, 16.0, 14.7 in NR9405. Remarkable difference exists not only between different germplasms but also among the leaves of different leaf locations within the same germplasm.

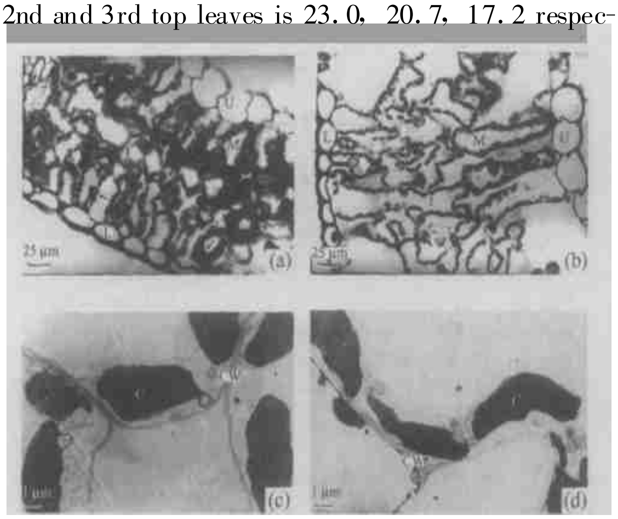

Fig. 3. Mesophyll cells and chloroplasts of low and high temperature germplasm. (a) Mesophyll cells in flag leaf of low temperature germplasm Shaan229. U, upper epidermis; L, lower epidermis; M, mesophyll cells; the arrow points to the chloroplast. The cells are smaller and closely arranged and chloroplasts are arranged more closely in multiple layers. (b) Mesophyll cells in flag leaf of high temperature germplasm NR9405. The symbols have the same meaning as those in (a). Cells are comparatively large and less closely arranged and chloroplasts are arranged in a single layer. (c) Chloroplasts in the 2nd top leaf of low temperature germplasm Shaan229. CW, cell wall; C, chloroplast. The chloroplast volume is comparatively large, and the short diameter is comparatively long. (d) Chloroplasts in the 2nd top leaf of high temperature germplasm NR9405. The symbols have the same meaning as those in (c). The chloroplast volume is comparatively small and the short diameter is comparatively short.

There are remarkable differences in the distribution of chloroplast stroma, grana and grana lamellae between the low and the high temperature germplasms. The former has thick stroma, more granas and well developed grana lamellae consisting of thylakoids (Fig. 4 (a)); yet the latter has thin stroma, fewer granas, and comparatively tenuous grana lamellae (Fig. 4 (b)).

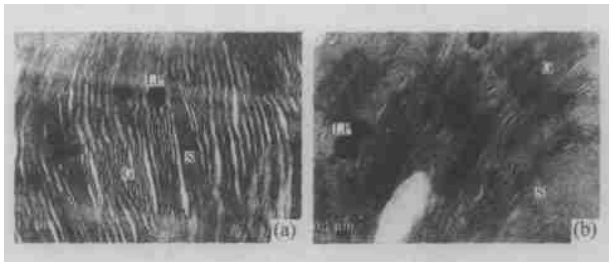

Fig. 4. Chloroplast granas and grana lamellar structure in the low and high temperature germplasm leaves. (a) Grana lamellar structure of the chloroplast in flag leaf of the low temperature germplasm Shaan229. S, stroma; G, grana and its structure; LP, osmiophilic globule. The stroma is thick, and there are more granas and a well developed lamellar structure. (b) Grana lamellar structure of the chloroplast in flag leaf of the high temperature germplasm NR9405. The symbols mean the same as those in (a). The stroma is thin, and there are fewer granas and a less developed lamellar structure.

The percentage of all lamellae of the granas in which the grana lamellar number of each grana is more than 20 in the flag leaf, the 2nd and 3rd top leaves of Shaan229 is 17.6%, 7.6% and 3.9% higher respectively than those of NR9405. However, the percentage of all lamellae of the granas in which the grana lamellar number of each grana is less than 10 in the flag leaf, the 2nd and 3rd top leaves of Shaan229 is 26.2%, 7.6% and 4.7% lower respectively than those of NR9405. These results indicate that the endomembrane system in chloroplasts of the low temperature germplasm is better developed than that of the high temperature germplasm, meaning that the low temperature germplasm has more organs to capture light energy effectively and accelerate photosynthesis.

Through the growth process, the differences in leaf structure between the low and the high temperature germplasm become greater. The high temperature germplasm shows signs of early senescence. Its mesophyll cells, chloroplasts and grana lamellae begin to decompose, and the electron density in the stroma of chloroplast and grana stacks decreases, and the osmiophilic globules increase in number and size. In contrast, the low temperature germplasm structure shows fewer signs of senescence, especially in the late milky stage. At this stage, in the high temperature germplasm, the leaf chloroplasts have decomposed completely and the grana thylakoids have also been destroyed completely, and there is almost no visible structure (Fig. 5 (a)), though the grains are still far from maturation. Yet in the low temperature germplasm, there are still chloroplasts in mesophyll cells of the flag leaf, although the 2nd and 3rd top leaves exhibit strong signs of senescence. Also, the grana lamellar structure is comparatively intact in the low temperature germplasm at this stage (Fig. 5 (b)).

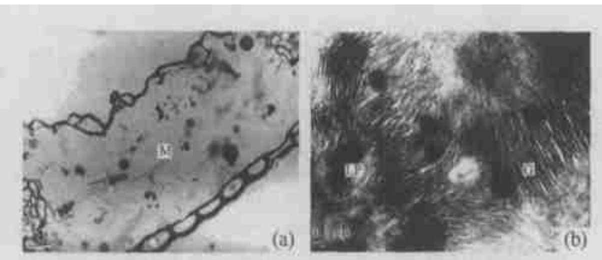

Fig. 5. Leaf structure of the low and high temperature germplasms in late grain filling stage. (a) Mesophyll cells in flag leaf of the high temperature germplasm NR9405. M, Mesophyll cell. Mesophyll cell and organelle decomposed, and nearly no visible structure. (b) Grana and grana lamellar structure in chloroplasts in the flag leaf of the low temperature germplasm Shaan229. G, Grana and its structure; LP, osmiophilic globule. Grana lamellar structure is comparatively intact.

2.2.4 Vascular bundles of leaf

Vascular bundles in the wheat leaf vary in size and are arranged alternately. Usually there are three small vascular bundles between the large ones. Cross-sectional area of the large and small vascular bundles in the flag leaf in particular can illuminate the differences between the low and high temperature germplasms in this regard (Table 2). The vascular bundle area refers to the area enclosed by the inner vascular bundle sheath.

Table 2 shows that the cross-sectional area of the big and small vascular bundles in flag leaves of the low temperature germplasm is larger than that of the high temperature germplasm. Statistical analysis shows that $T = 15$, and $T_2 = 15$ ($\alpha = 0.05$), and $T \geqslant T_2$. This indicates that there are significant differences between the low and high temperature germplasms with respect to the cross-sectional area and weighted average of the large and small vascular bundles in flag leaves. Vascular bundle state of the 2nd and 3rd top leaves of the low and high temperature germplasms is similar to that of flag leaves.

Table 2. Cross-sectional area of big and small vascular bundles in flag leaves of the low and high temperature germplasms (μm^2)

Vascular bundle type	Low temperature germplasm			High temperature germplasm		
	Shaan229	Xiaoyan6	901	NR9405	9430	Yanshi9
Big vascular bundle	5497	5325	6528	4397	4412	4412
Small vascular bundle	1649	1546	1472	1325	1020	1236
Weighted average	2611	2491	2736	2093	1868	2030

Statistical results indicate that the interval between vascular bundles in three top leaves of the low temperature germplasm is smaller than that of the high temperature germplasm. The higher the leaf location, the smaller the interval. This means that the low temperature germplasm leaf not only has larger vascular bundle cross-sectional area but also has more vascular bundles per leaf width. This not only favors the transportation of water and mineral salts from soil to different leaf locations in time, but also favors the transportation of photosynthetic substances from leaves to other plant organs, resulting in an enhanced photosynthetic rate.

Further observation of the ultrastructure of vascular bundles in the low temperature germplasm reveals that there are well-developed plasmodesma between two layers of vascular bundle sheath cells, between inner vascular bundle sheath cells and parenchymatous cells, and between parenchymatous cells of vascular bundles, between sieve tubes and companion cells. This further enhances the transportation of photosynthetic substances and accelerates the photosynthetic process. However, these characteristics are less developed in high temperature germplasm.

3 Discussion

The analysis above indicates that the chlorophyll content and net photosynthetic rate of the low and high temperature germplasms increase with ascending of leaf location and that, with this ascent, leaf structure tends to become more complex. The number of mesophyll cells per unit area leaf and the chloroplasts in each mesophyll cell increase and the interval between vascular bundles diminishes with ascending of leaf location. These results agree with those of earlier studies[14,15], which also indicate a consistency between mesophyll cell structure complexity and photosynthesis improvement.

The relationship between leaf structure and function at different leaf locations within the same genotype is further elucidated through comparison of wheat genotypes with different plant temperatures. Low temperature germplasm leaves have relatively high chlorophyll content and net photosynthetic rate. Mesophyll cells are small and closely arranged with more layers. Chloroplasts are dense and have thick stroma, many granas and well developed grana lamellae. Conductive tissues are also better developed. In contrast, the high temperature germplasm exhibits weaker leaf structure and photosynthetic ability. In producing these results, this research confirms the consistency between morphological structure and physiological function of leaves, and importantly explains the high photosynthetic traits of the low temperature germplasm leaves in terms of structure.

The low temperature wheat is one of the few low temperature plant materials found recently, but its good function and structure should stimulate researchers to find and cultivate more low temperature materials. Such work will not only promote current studies on other low temperature plants but also contribute much to the yield improvement of economic plants.

References

1 Blum, A. et al. Infrared thermal sensing of plant canopies as a screening technique for dehydration avoidance in wheat. Field Crops Research, 1982, 5: 137.

2 Harris D. S. et al. Genetic diversity in soybeans for leaf canopy temperature and the association of leaf canopy temperature and yield. Crop Science, 1984, 24: 839.

3 Mckinney, N. V. et al. Canopy temperature, seed yield, and vapor pressure deficit relationship in soybeen. Crop Science, 1989, 29(4): 1038.

4 Zhang, S. W. The second heat source of wheat population and its heating effects. Journal of Ecology (in Chinese), 1990, 9(2): 1.

5 Rees D. et al. Canopy temperature of wheat: Relationship with yield and potential as a technique for early generation selection. In: Wheat Special Report, No. 36 (ed. CIMMYT), Mexico, 1993, 32.

6 Frank, A. B. et al. Carbon isotope discrimination, ash, and canopy temperature in three wheatgrass species. Crop Science, 1997, 37(5): 1573.

7 Singh, N. B. et al. High temperature tolerance in wheat cultivars. Advances in Agricultural Research in India, 1997, 7: 119.

8 Ranalli, P. et al. Drought tolerance screening for potato improvement. Plant Breeding, 1997, 116(3): 290.

9 Alza, J. O. et al. Genetic analysis of yield and related traits in sunflower (*Helianthus annuus* L.) in dryland and irrigated environments. Euphytica, 1997, 95(2): 243.

10 Reynolds M. P. et al. Evaluating physiological traits to complement empirical selection for wheat in warm environments. Developments in Plant Breeding, 1997, 6: 143.

11 Fischer, R. A. et al. Wheat yield progress associated with higher stomatal conductance and photosynthetic rate, and cooler canopies. Crop Science, 1998, 38(6): 1467.

12 Rashid, A. et al. Use of canopy temperature measurements as a screening tool for drought tolerance in spring wheat. Journal of Agronomy and Crop Science, 1999, 182(4): 231.

13 Zhang, S. W. et al. Some traits of low temperature germplasm wheat under extremely unfavorable weather conditions. Progress in Natural Science, 2001, 11(12): 911.

14 Duan, X. C. et al. Study on the leaf cells of wheat—the ontogeny of winter wheat and variations in the structure of the mesophyll cell and many other types of cells. Acta Botanica Sinica, 1974, 16(3): 254.

15 Zheng, P. Y. et al. Studies on the morphology and function of the mesophyll cells of leaf-blades and leaf-sheaths in Rye (*secale cereale* L.). Acta Agronomica Sinica, 1993, 19(1): 29.